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1: Cell Stress Chaperones. 1998 Mar;3(1):6-11.

[Related Articles, Links](#)**Definition of extracellular localized epitopes of Hsp70 involved in an NK immune response.****Botzler C, Li G, Issels RD, Multhoff G.**

GSF-Institute of Clinical Hematology and Klinikum Grosshadern, Med. Klinik III, Munich, Germany.

In order to define extracellular localized epitopes of Hsp70 on human tumor cells which are accessible to the immune system, six commercially available Hsp70-specific monoclonal antibodies (mAb) with different recognition sites were examined by immunological approaches. The recognition pattern of these antibodies was analyzed on purified recombinant Hsp70 proteins (rHsp70, Hsc70, DnaK), on lysates of Hsp70-expressing colon carcinoma cells (CX+) and on lysates of M21 rat-1 cells that overexpress human Hsp70 or Hsp70 fragments: ABgl (del 120-428) consisting of the C-terminal part and ASma (del 438-618) consisting of the N-terminal part of human Hsp70. All antibodies reacted equally well with rHsp70 and cytoplasmic Hsp70 derived from human tumor cells or M21 rat-1 cells. Only one antibody (MA3-007; Hsp70, Hsc70) detects a region localized within the ATPase domain of Hsp70 (amino acid 122-264) and reacts positively with the C-terminal deletion mutant ASma. All other antibodies, including RPN1197 are directed against the C-terminal peptide binding domain of Hsp70 and react positively with the N-terminal deletion mutant ABgl. Although all six antibodies detect full-length Hsp70 protein, derived from plasma membrane fractions of CX+ tumor cells, cell surface expressed Hsp70 on viable CX+ tumor cells, as determined by flowcytometry, is only recognized with the antibodies MA3-006 (Hsp70, Hsc70; 504-617), MA3-009 (Hsp70; 504-617) and RPN1197 (Hsp70). An estimation of the ratio of membrane-bound to cytoplasmic Hsp70 molecules revealed that 15-20% of total Hsp70 molecules are expressed on the plasma membrane. This tumor-selective cell surface expression of Hsp70 correlates with an increased sensitivity to lysis mediated by non-MHC restricted natural killer (NK) cells. We demonstrate that only antibodies directed against membrane-bound Hsp70 (MA3-006, MA3-009, RPN1197) inhibit NK-killing activity against Hsp70-expressing tumor cells. Taken together our data indicate that at least the C-terminal

region 504-617, that contains at least one single alpha-helix (amino acid 512-536), has to be localized extracellularly and might be of importance for an NK-mediated anti-tumor immune response.

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